# Synthesis of GnRH and Somatostatin Cytotoxic Drug Conjugates


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**Booklet of the PhD thesis**

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## 1. Introduction

Cancer is the second leading cause of death worldwide and was responsible for more than 9 million deaths in 2018 [1]. Targeted tumor therapy is a promising tool to overcome the drawbacks of traditional chemotherapy, like the lack of selectivity and harmful side effects to healthy tissue. The basis of this approach is the overexpression of tumor specific cell surface proteins, like peptide hormone receptors. Natural ligands or related high affinity binders of these receptors are promising targeting moieties for the selective delivery of cytotoxic agents to tumor cells. Prominent examples are the two peptide hormones, gonadotropin-releasing hormone (GnRH) and somatostatin (SST). Both hormones are of high clinical relevance and their synthetic analogs are used as therapeutics for the treatment of hormone related cancer [2]. Considering that GnRH and SST analogs exert not only an indirect antitumor activity by inducing hormonal dysfunctions, but also elicit a direct antiproliferating activity by binding to their highly expressed receptors on tumor cells, both peptide hormones are valuable candidates for the development of drug delivery systems (DDS). The first cytotoxic GnRH and SST derivatives have been developed in the 1980s, whereby intensive preclinical studies illustrate the high value of doxorubicin (Dox) and 2-pyrrolino-Dox (pyDox)-linked GnRH and SST derivatives [3–5].

Next to human GnRH (GnRH-I), the natural sea lamprey isoform GnRH-III (Glp-His-Trp-Ser-His-Asp-Trp-Lys-Pro-Gly-$NH_2$, Glp is pyroglutamic acid) represents a promising homing device for GnRH-based drug delivery systems (DDS). This weak GnRH agonist binds to GnRH-receptors (GnRH-R) on cancer cells and induces a direct antitumor activity, while its endocrine effect is strongly reduced compared to GnRH-I [6]. Based on these findings, a huge variety of GnRH-III-based DDS have been developed in our laboratories where daunorubicin (Dau) was linked to the lysine side chain of GnRH-III *via* oxime bond formation to an inserted aminooxyacetyl (Aoa) moiety [7–10]. It could be shown that these conjugates are highly stable in circulation and exert a substantial growth inhibitory effect on cancer cells. Although this linker system prevents the intracellular release of the free drug, it was demonstrated that lysosomal enzymes ensure the formation of active drug-containing metabolites which are able to intercalate into DNA strands and thus, inhibit macromolecule biosynthesis which is accompanied with reduced cell proliferation [8]. Moreover, the results pointed out that the DNA binding affinity is slightly diminished in comparison to the free Dau. In the recent years, different systematical refinement studies have been performed to improve the antitumor activity of the oxime bond-linked GnRH-III-Dau conjugates, resulting in our lead compound GnRH-III-[$^4$Lys(Bu),$^8$Lys(Dau=Aoa)] (**K2**) (where Lys(Bu) is butyrylated lysine) which induces a

strong *in vitro* and *in vivo* antitumor activity on GnRH-R expressing cells [10,11].

Apart from that, synthetic derivatives of SST, a cyclic neuroendocrine peptide, have been used as carriers to deliver cytotoxic drugs to SST receptor expressing cancer cells. Next to the market approved SST analogs octreotide and lanreotide, two other SST compounds, namely RC-121 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-$NH_2$) and TT-232 (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-$NH_2$) (cyclized by disulfide bond), provide valuable benefits for targeted tumor therapy [12,13]. RC-121 possesses a highly enhanced potency and longer duration of action for inhibition of growth hormone release than native SST [12]. Moreover, it exhibits a significant *in vitro* and *in vivo* inhibitory effect on different cancer cells and has been successfully used as peptide carrier for drug delivery [4,5]. Considering that all 5 naturally occurring SST receptors (SSTR1-5) are also present with varying incidence on tumor cells, a selective targeting of one or two SSTRs would be favorable. It could be shown that RC-121 binds with high affinity to SSTR2 and with moderate affinity to SSTR5, while TT-232 displayed the highest affinity for SSTR4, followed by moderate affinity to SSTR1 and SSTR5 [5,13]. In comparison to RC-121, TT-232 has no growth hormone release inhibitory activity, but exerts a strong *in vitro* and *in vivo* antineoplastic activity on a wide range of malignant tumors, including breast, prostate and colon cancer [13]. Therefore, TT-232 represents a valuable candidate for targeted tumor therapy without causing undesirable endocrine effects.

**2. Aims and objectives**

Based on these research findings, the central goal of my thesis was the development and evaluation of GnRH- and somatostatin-based drug delivery systems for targeted tumor therapy. Moreover, certain main objectives have been defined:

1. Improvement of the antitumor activity of oxime bond-linked GnRH-III-Dau conjugates:
   - synthesis of oxime bond-linked GnRH-III-Dau conjugates, using solid phase peptide synthesis (SPPS) and ligation of Dau in solution and evaluation of the cytostatic effect of the compounds in comparison to **K2** by cell viability assays
   - additional analyses of (best) candidates to analyze the cellular uptake (flow cytometry) and localization (confocal laser scanning microscopy (CLSM)), stability in plasma and in presence of lysosomal enzymes (LC-MS assay) and GnRH receptor affinity by radio ligand binding studies in order to validate the results of the cell viability assays and to prove the mechanism of action of the conjugates

2. Development of cleavable linker containing GnRH-drug conjugates:
   - synthesis of targeting moieties by SPPS (best carriers from the 1. objective) and synthesis

of paclitaxel (PTX) and Dau-containing linker systems in solution consisting of Val-Ala or Val-Cit cathepsin B cleavage site and a *para*-aminobenzyloxycarbonyl (PABC) self-immolative spacer, followed by attachment to carrier

- synthesis of non-cleavable counterparts as controls
- evaluation and comparison of cytostatic effect of the conjugates to gain information about the impact of the linker system
- proof of linker concept by lysosomal degradation studies and receptor binding studies

3. Comparison of different SST carriers and linker systems to establish a new SST lead compound:

- synthesis of 5(6)-carboxyfluorescein (FAM) labeled SST-compounds by SPPS and cellular uptake studies of the FAM compounds by CLSM and flow cytometry
- synthesis of equivalent oxime bond-linked SST-Dau conjugates and analysis of the cytostatic effect to select the best targeting moiety
- synthesis of additional SST-conjugates with different linker systems and evaluation of their cytostatic effect to choose the most promising one
- synthesis of 2-pyrrolino-Dau (pyDau) SST conjugate using the best carrier-linker combination and evaluation of the antitumor activity

3. **Methods**

The GnRH-III and SST peptide moieties were synthesized by solid phase peptide synthesis (SPPS) according to Fmoc/tBu chemistry on Rink-Amide MBHA or Fmoc-Ethyl-Indole AM resin and the oxime bond ligation was carried out in 0.2 M $NH_4OAc$ buffer and stirred overnight, whereby the carbonyl group of Dau was used for conjugation *via* oxime bond formation [10]. The SST-derivatives were cyclized either by disulfide bond formation (air oxidation) or thioether bond formation in aqueous, alkaline buffer.

For the synthesize of cleavable GnRH-drug conjugates, dipeptidyl-PABC-drug linkers were prepared in solution as recently reported [14,15]. Dau and PTX were used as payloads. The amino group of the Dau sugar was used as ligation site to connect the PABC moiety by carbamate group formation. PTX was linked to its C2'-OH group by carbamate group formation to an *N*,*N'*-dimethylethylenediamine (here further named as diamine) spacer. This spacer was attached to the PABC moiety. A glutaryl spacer was added to the *N*-terminus to facilitate the conjugation of the linker to $^{8}$Lys of the GnRH-III carriers by amide bond formation, affording glutaryl-Val-Aaa-PABC-Dau and glutaryl-Val-Aaa-PABC-diamine-PTX linkers. Besides,

non-cleavable linkers have been also synthesized, namely glutaryl-Dau and glutaryl-diamine-PTX. Each linker was coupled to both peptide carriers affording 12 novel GnRH-III conjugates.

All conjugates were purified by preparative RP-HPLC and characterized by analytical HPLC and ESI-MS.

The cytostatic effect of the conjugates was determined by resazurin- or MTT-based cell viability assay. Therefore, cells were treated for 24 hours (Dau-conjugates) or 6 hours (PTX-conjugates) one day after seeding, followed by wash out of the compounds. The cell viability was determined 72 hours after treatment initiation.

The cellular uptake of the compounds was investigated by flow cytometry or CLSM. For both experiments, the cells were treated for 6 hours. Flow cytometry studies were performed on a BD LSR III flow cytometer. For the CLSM studies, cells were seeded on cover glasses. After treatment period, the cover glasses were mounted to microscopy slides. The images were recorded on a Zeiss LSM 710 system

The stability in human plasma and the lysosomal degradation studies in presence of rat liver lysosomal homogenate were performed as recently described [8]. The sample analysis was performed by LC-MS.

The radioligand binding studies with radiolabeled triptorelin (GnRH-I-[$^{6}$D-Trp]) were measured in the same manner as recently reported [10].

**4. New results**

*4.1. GnRH-drug conjugates*

*Improvement of the antitumor activity of oxime bond-linked GnRH-III-Dau conjugates*

1. We synthesized and analyzed novel GnRH-III derivatives with unnatural amino acids (Aaa) within the peptide sequence. Two different strategies have been followed yielding two different sets of GnRH-III-Dau conjugates:

- 1$^{st}$ set of GnRH-III-Dau conjugates: $^{6}$Asp was substituted by D-Asp, D-Glu and D-Trp.
- 2$^{nd}$ set of GnRH-III-Dau conjugates: this set comprises different changes, including modification of the *C*-terminus from -Pro-Gly-$NH_2$ to -Pro-ethylamide (EA), methylation of $^{6}$Asp to $^{6}$Asp(OMe), $^{3}$Trp exchanged by $^{3}$D-Trp or $^{3}$D-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid), as well as the combination of $^{3}$D-Tic with $^{7}$D-Trp and all $^{3}$D-Tic-conjugates in combination with $^{2}$His deletion.

2. All GnRH-III-Dau-conjugates of the 1$^{st}$ set revealed a substantial growth inhibitory effect on estrogen-dependent breast and reproductive system unrelated colon cancer cells, but none of

the novel $^{6}$D-Aaa GnRH-III conjugates revealed a higher biological activity than our lead compound **K2**.

3. Based on further experiments, we could demonstrate that the anticancer activity of the conjugates strongly depends on the cellular uptake and the efficient release of the small drug metabolites by lysosomal enzymes.

4. CLSM studies were used to verify the endocytic uptake of our lead compound **K2**. Time-dependent uptake studies pointed out that Dau reached its site of action already within 10 minutes treatment period which was validated by co-staining of the nuclei. At lower time points, the Dau-signal was predominantly detected in small cytosolic vesicles which were proposed to be lysosomes or endosomes. This assumption could be confirmed by co-staining of the lysosomes.

5. The GnRH-R mediated uptake could be further verified by performing a receptor competition assay with GnRH-I superagonist triptorelin. We could also show that the use of increasing triptorelin concentrations and constant concentration of **K2** reduce the cellular uptake rate of **K2** substantially.

6. Considering that the first modifications did not result in the desired improvement of the antitumor activity, further modifications have been applied and the growth inhibitory effect of the compounds was studied initially on human estrogen dependent MCF-7 breast and hormone independent HT-29 colon cancer cells. The results revealed a clearly improved anticancer activity of compound GnRH-III-[$^{2}$ΔHis,$^{3}$D-Tic, $^{4}$Lys(Bu), $^{8}$Lys(Dau=Aoa)] (**16**) on both cell lines, whereby this effect was higher on breast cancer cells. Furthermore, the activity of **K2** and **16** was investigated on estrogen independent MDA-MB-231 breast cancer cells. We obtained an improved anticancer activity of **16** over **K2** also on this cell line, indicating that the activity was not exclusively increased on estrogen dependent breast cancer cells.

7. In order to better interpret these results, a variety of comparable studies between **K2** and **16** have been performed. Hence, the degradation of **K2** and **16** by lysosomal enzymes was analyzed, demonstrating that the *N*-terminal region of **16** shows a higher durability towards lysosomal proteases. However, the release of the smallest active metabolite (H-Lys(Dau=Aoa)-OH) could be obtained for both conjugates within the first hour, since the degradation of the *C*-terminus was not affected which might be of high relevance for the biological activity of the oxime bond linked GnRH-III-Dau conjugates.

8. Cellular uptake studies on MCF-7 breast and HT-29 colon cancer cells by flow cytometry pointed out that compound **16** was taken up more efficiently than **K2**. This effect could be

particularly demonstrated in case of smaller concentrations of the conjugates. Moreover, the uptake rates were higher on estrogen dependent breast cancer cells than on colon cancer cells, being in line with the results of the cytostatic effect analysis. Moreover, we demonstrated that the receptor binding affinity of **16** is slightly better than that of **K2**. According to these results, it can be assumed that the improved anticancer activity is related to an enhanced cellular uptake of **16**.

9. The time-dependent cellular uptake of compound **16** was studied by CLSM. We could detect the Dau-signal already after 5 minutes of treatment period in nuclei which was faster than the delivery of Dau to its site of action by **K2** (see above), revealed in comparable studies. Taking this into account, it can be proposed that the improved cellular uptake of **16** is accompanied with an accelerated delivery of the drug.

10. The stability of both compounds was measured in presence of human and mouse blood plasma, whereby no degradation could be obtained within 24 hours incubation at 37 °C. This is of great importance for the stability of the compounds in circulation and the selective delivery of the compounds to cancerous, GnRH-R expressing cells. Moreover, the high durability in mouse plasma provides a good basis for upcoming *in vivo* studies on tumor bearing mice.

*Development of cleavable linker containing GnRH-drug conjugates*

1. We established the synthesis route of cleavable self-immolative linker-containing GnRH-Dau and -PTX conjugates and related non-cleavable compounds. Based on the results of **K2** and **16**, the corresponding peptide carriers were used as targeting moiety. To ensure the release of the free drug, self-immolative PABC linker systems with cathepsin B cleavable sites (Val-Ala or Val-Cit) were used to link the drugs.

2. We determined the growth inhibitory effect of the conjugates on human A2780 ovarian cancer cells which highly express GnRH-Rs, and Panc-1 pancreatic cancer cells, revealing a lower level of GnRH-R expression. All cleavable linker containing conjugates displayed an effective cell growth inhibition on ovarian cancer cells, while the activity of the non-cleavable linker derivatives was strongly reduced, indicating the high value of the self-immolative linker systems. Moreover, we could show that the antitumor activity on pancreatic cells was reduced by approximately one order of magnitude, compared to the results obtained on ovarian cancer cells which seems to be caused by the selectivity of the compounds towards GnRH-R expressing cells. Furthermore, we could demonstrate that the Dau-compounds with the new targeting sequence revealed higher activities than the compounds with the **K2-**derived peptide carrier which further underlines the valuable character of the novel GnRH-targeting moiety. In

contrast, we detected comparable cell growth inhibitory effects for the cleavable PTX-conjugates. The direct comparison of the oxime bond-linked Dau-compounds **K2** and **16** showed that both compounds have a slightly improved anticancer activity on GnRH-R expressing ovarian cancer cells than the self-immolative counterparts.

3. We could validate the releasing concept of the self-immolative linkers by lysosomal degradation of distinct Dau and PTX conjugates. Thus, we demonstrated that Dau was efficiently released from both linker systems within the first hour, whereby the release was slightly faster for the Val-Cit-linker. In comparison, the liberation of Dau could not be detected for the non-cleavable linkers. These results clearly prove the releasing concept of the Dau-conjugates indicating that the slightly reduced activity is not related to an insufficient Dau release. In case of the PTX conjugates, we could detect the release of the diamine-PTX fragment for both cleavable linker systems already after 5 minutes of incubation, but the release of the free drug was not observed within 24 hours of incubation which might explain the similar antitumor activities of the compound. In case of the non-cleavable PTX conjugate, the release of the diamine-PTX was not detected.

4. The GnRH-receptor binding affinities of the cleavable GnRH-III Dau derivative with the best anticancer activity and its PTX equivalent have been determined, revealing that the affinity of the cleavable Dau-derivative was reduced by a factor of 7 compared to compound **16**, while the affinity of the PTX-compound was 4-times reduced. This leads to the assumption that the decreased receptor binding affinity has a larger influence on the biological activity than the reduced DNA binding properties of the smallest Dau-containing metabolite of the oxime bond Dau conjugates.

*4.2. Somatostatin conjugates*

*Comparison of different SST carriers and linker systems to establish a new SST lead compound*

*FAM-labeled somatostatin conjugates*

1. To compare the two SST targeting moieties RC-121 and TT-232 with a novel targeting moiety which possesses the same ring size as RC-121, but is cyclized by a thioether bond, we synthesized fluorescently labeled compounds.

2. Flow cytometry studies showed that the RC-121 targeting moiety was taken up most efficiently on breast, as well as colon cancer cells, followed by the TT-232 derivative and then the thioether compound. Moreover, we could show that the incorporation of a hydrophilic linker has a positive impact on the uptake rate of the compounds.

3. The images of the CLSM studies on breast cancer cells displayed strong fluorescent signals in the cytosol for the linker containing compounds, while in case of the conjugates without linker, cytosolic vesicles have been predominantly detected. According to these results, we can conclude that the LRRY-spacer did not only enhance the cellular uptake, but also ensured the release of FAM-containing fragments, whereas the *N*-terminal D-Phe of the compounds without linker prevents this release.

*Somatostatin-drug conjugates*

1. We developed comparable oxime bond-linked Dau=Aoa-LRRY-derivatives and determined their cytostatic effect on human colon cancer and estrogen dependent breast cancer cells. These studies pointed out that the RC-121-Dau compound was the most efficient on both cell lines. On the contrary, the TT-232-Dau conjugate revealed a low cytostatic activity on colon cancer cells, while its activity was clearly improved on breast cancer cells. The thioether bond containing Dau-conjugate revealed comparable activities on both cell lines, thereby it was more effective on colon cancer cells and less active on breast cancer cells than the TT-232-Dau conjugate. Due to these results, we assume that breast cancer cells express a higher amount of SSTR4 than colon cancer cells, while SSTR2 expression seems to be similar on both cell lines.

2. We established the synthesis of two new RC-121-Dau conjugates with different linker systems, one linker system was Dau=Aoa-LRRYC-$NH_2$ which was attached by thioether bond formation to an *N*-terminally inserted chloroacetyl moiety of RC-121 and the other was glutaryl-Val-Ala-PABC-Dau which revealed the best activity in the GnRH study. This linker was directly coupled to the *N*-terminal D-Phe of RC-121 by amide bond formation.

3. In direct comparison on human breast and colon cancer cells, the initial oxime bond-linked Dau=Aoa-LRRY-RC-121 compound was again evaluated as the conjugate with the highest anticancer activity, directly followed by the self-immolative linker containing compound. The thioether linker derivative showed a lower growth inhibitory effect than the other two compounds and a slightly better effect than the novel thioether cyclized derivative of the initial study.

4. These results prompted us to ligate the highly potent Dau derivative pyDau to the Aoa-LRRY-RC-121 peptide by oxime bond formation. The resulting compound displayed a 20-35-times improved growth inhibitory effect on breast and colon cancer cells. This strong anticancer activity demonstrates the high potential of pyDau-based DDS for targeted cancer therapy.

## 5. Summary

Since many cancer cells overexpress receptors for the peptide hormones GnRH and somatostatin, their ligands can be used as homing devices to deliver cytotoxic cargos selectively to cancer cells. Hence, the present thesis deals with the synthesis and biochemical characterization of novel GnRH-III and SST-drug conjugates.

A variety of GnRH-III-Dau conjugates have been established and systematically refined in our research group. To achieve an improved antitumor activity of oxime bond-linked GnRH-III-Dau compounds, 20 novel conjugates with modified peptide sequence have been prepared. The *in vitro* cytostatic effect of these derivatives was studied on GnRH-R expressing cancer cells and compared to our lead compound **K2**. The conjugate GnRH-III-[$^{2}$ΔHis-$^{3}$D-Tic-$^{4}$Lys(Bu), $^{8}$Lys(Dau=Aoa)] (**16**) displayed a significantly improved antitumor activity. Besides, cellular uptake and localization studies, stability analysis in plasma and degradation by lysosomal enzymes, as well as receptor binding studies have been performed. We could show that the increased biological activity is mainly related to the improved cellular uptake of **16**. Moreover, the results of the studies underline the high potential of **16** for targeted tumor therapy.

Further GnRH-III-drug conjugates were established using the best targeting moieties. The anti-cancer drugs PTX and Dau were linked to the peptides using cathepsin B labile, self-immolative linkers. Cell viability studies on human cancer cells verified the cytostatic effect of the cleavable GnRH-III derivatives. Moreover, the drug releasing concept was validated by lysosomal degradation studies. In summary, we could show that next to the release of the free drug, the receptor binding affinity and the cellular uptake are very important factors for the anticancer activity.

In addition, SST-drug conjugates were prepared and evaluated. Initially, the potential of different targeting moieties was analyzed. Thus, FAM-labeled derivatives were developed to determine the cellular uptake of the compounds. Due to these results, related oxime bond-linked Dau-conjugates were synthesized, and the cytostatic effect was studied on SSTR expressing cancer cells. The best targeting moiety was selected and used to study the influence of distinct linker systems on the anticancer activity. The best candidate consists of the RC-121 carrier and the drug linker Dau=Aoa-LRRY. To further enhance the antitumor activity, the highly potent anticancer drug 2-pyrrolino-daunorubicin was used instead of Dau affording conjugate pyDau=Aoa-LRRY-RC-121 (**71**) which revealed a strongly increased *in vitro* anticancer activity. Thus, this SST conjugate is a highly promising candidate for targeted cancer therapy.

Our results confirm the high potential of compound **16** and **71** for selective cancer therapy which underlines the great value of GnRH-III and SST-drug conjugates for targeted tumor therapy.

## 5. Publication list

*Publications in frame of the PhD project*

**Schuster, S**.; Biri-Kovács, B.; Szeder, B.; Farkas, V.; Buday, L.; Szabó, Z.; Halmos, G.; Mező, G. Synthesis and in vitro biochemical evaluation of oxime bond-linked daunorubicin–GnRH-III conjugates developed for targeted drug delivery. *Beilstein J. Org. Chem*. **2018**, *14*, 756–771. [doi:10.3762/bjoc.14.64]

**Schuster, S.;** Biri-Kovács, B.; Szeder, B.; Buday, L.; Gardi, J.; Szabó, Z.; Halmos, G.; Mező, G. Enhanced In Vitro Antitumor Activity of GnRH-III-Daunorubicin Bioconjugates Influenced by Sequence Modification. *Pharmaceutics.* **2018**, *10*(4), 223-242.

*Further publications*

**Schuster, S**.; Roessler, C.; Meleshin, M.; Zimmermann, P.; Simic, Z.; Kambach, C.; Schiene-Fischer, C.; Steegborn, C.; Hottiger, M.O.; Schutkowski, M. A continuous sirtuin activity assay without any coupling to enzymatic or chemical reactions. *Sci Rep*. **2016**, *6*, 22643. [doi:10.1038/srep22643]

*Conference proceeding in frame of the PhD project*

**Schuster, S**.; Biri-Kovács, B.; Borbély, A.; Sewald, N.; Neundorf, I.; Gennari, C.;, Mező, G. Synthesis and biochemical evaluation of GnRH-III drug conjugates. *In: Patrick B. Timmons, Chandralal M. Hewage, Michal Lebl (eds) Proceedings of 35th European Peptide Symposium*. **2018**. 80-83

**Schuster, S.;** Biri-Kovács, B.; Mező, G. Influence of sequence modification in GnRH-III on the efficiency of tumour targeting. *In: A. Beck-Sickinger, K. Mörl, K. Bellmann-Sickert, S. Els-Heindl, U. Diederichsen (eds.) Proceedings of 34th European Peptide Symposium*. **2017**. 167-168

## 9. References


[1] "WHO | Cancer," can be found under http://www.who.int/cancer/en/, **n.d.**
[2] G. Mezo, in *Amino Acids Pept. Proteins*, **2013**, pp. 203–252.
[3] A. Nagy, A. V. Schally, *Biol. Reprod.* **2005**, *73*, 851–859.
[4] A. V. Schally, A. Nagy, *Life Sci.* **2003**, *72*, 2305–2320.
[5] G. Mező, M. Manea, *Expert Opin. Drug Deliv.* **2010**, *7*, 79–96.
[6] M. Kovacs, J. Seprodi, M. Koppan, J. E. Horvath, B. Vincze, I. Teplan, B. Flerko, *J. Neuroendocrinol.* **2002**, *14*, 647–655.
[7] I. Szabó, M. Manea, E. Orbán, A. Csámpai, S. Bősze, R. Szabó, M. Tejeda, D. Gaál, B. Kapuvári, M. Przybylski, et al., *Bioconjug. Chem.* **2009**, *20*, 656–665.
[8] E. Orbán, G. Mezo, P. Schlage, G. Csík, Z. Kulić, P. Ansorge, E. Fellinger, H. M. Möller, M. Manea, *Amino Acids* **2011**, *41*, 469–483.
[9] M. Manea, U. Leurs, E. Orbán, Z. Baranyai, P. Öhlschläger, A. Marquardt, Á. Schulcz, M. Tejeda, B. Kapuvári, J. Tóvári, et al., *Bioconjug. Chem.* **2011**, *22*, 1320–1329.
[10] R. Hegedüs, M. Manea, E. Orbán, I. Szabó, É. Kiss, É. Sipos, G. Halmos, G. Mező, *Eur. J. Med. Chem.* **2012**, *56*, 155–165.
[11] B. Kapuvári, R. Hegedüs, Á. Schulcz, M. Manea, J. Tóvári, A. Gacs, B. Vincze, G. Mező, *Invest. New Drugs* **2016**, *34*, 416–423.
[12] R. Z. Cai, B. Szoke, R. Lu, D. Fu, T. W. Redding, A. V. Schally, *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83*, 1896–1900.
[13] Z. Helyes, E. Pintér, J. Szolcsányi, *Drugs Future* **2005**, *30*, 558–566.
[14] A. Dal Corso, M. Caruso, L. Belvisi, D. Arosio, U. Piarulli, C. Albanese, F. Gasparri, A. Marsiglio, F. Sola, S. Troiani, et al., *Chem. – Eur. J.* **2015**, *21*, 6921–6929.
[15] G. M. Dubowchik, R. A. Firestone, L. Padilla, D. Willner, S. J. Hofstead, K. Mosure, J. O. Knipe, S. J. Lasch, P. A. Trail, *Bioconjug. Chem.* **2002**, *13*, 855–869.